

**REMARKS**

Claims 4, 9-10, 15-16 and 31-35 are currently pending in this application. Claims 4 and 31 have been amended. Support for the Amendment of claims 4 and 31 is found on page 3, lines 15 *et seq.* No new matter has been added. In view of these amendments and of the following remarks, Applicants believe that all the asserted rejections should be withdrawn and that all pending claims 4, 9-10, 15-16 and 31-35 are in condition for allowance.

Claims 4, 9-10, 15-16 and 31-35 stand rejected under 35 U.S.C. 103(a) for purported obviousness over Palva et al. and common knowledge in the art. Claims 4 and 31 have been amended to recite that the at least one restriction site is in the 3' terminus. Applicants respectfully refer the Examiner to the attached expert's Declaration Under 37 C.F.R. § 1.132, in which the Declarant attests that it is well known that not only is nucleotide sequence extremely important for promoter expression, but the length of the nucleotide sequence, or the number of bases, is extremely important as well.

The Declarant states that it is well known in the art that conservation of promoter structure in *Bacillus subtilis*, which is related closely to *Bacillus amyloliquefaciens* of the present invention, is much stricter than what is observed generally, for example in *E. coli*. Moreover, *B. subtilis* RNA polymerase demands high fidelity to the canonical -35 and -10 promoter hexanucleotides, and the *B. subtilis* ribosome may require extensive complementarity to messenger RNA.

The present invention modifies the promoter of  $\alpha$ -amylase derived from *B. amyloliquefaciens* by extending the Shine-Delgarno sequence by three nucleotides and by inserting at least one restriction site in the 3' end region. The Declarant states that these novel modifications result in new and unexpected results. The Declarant points to the evidence that is provided in Table 1 on page 18 of the specification. In recombinant *B. subtilis* microorganisms having an MPase gene, the productivity for MPase was increased over four-fold by using an  $\alpha$ -amylase promoter having at least one restriction site in the 3' end: MPase activity in units/l was 240,000 for the restriction enzyme sites BamHI and 240,000 for BamHI, SmaI, KpnI, SacI and EcoRI, whereas *B. subtilis* microorganisms without at least one restriction site in the 3' end, MPase activity in units/l was 50,000. In

recombinant *B. subtilis* microorganisms having a TPase gene, the productivity for TPase was increased over four-fold by using an  $\alpha$ -amylase promoter having at least one restriction site in the 3' end: TPase activity in units/l was 300,000 for the restriction enzyme sites BamHI and 300,000 for BamHI, SmaI, KpnI, SacI and EcoRI, whereas *B. subtilis* microorganisms without at least one restriction site in the 3' end, MPase activity in units/l was 70,000.

Palva et al. disclose the full sequence of the promoter derived from *Bacillus amyloliquefaciens* in Fig. 2, third line from the top: 5'-G AGA GGG AGA GGA AAC-3'. The sequence G AGA GGG AGA GGA from the 5' end, indicated by the solid and broken line in Fig. 2, constitutes a Shine-Dalgarno sequence, which is believed to be a potential RNA polymerase recognition site. The sequence 5'-G AGA GGG AGA GGA AAC-3' disclosed by Palva et al. contains the 10 nucleotides from the 3' end (underlined nucleotides) that are modified in the present invention. The Declarant states that, based on the above requirement that the conservation of the promoter structure in *B. subtilis*, i.e., *B. amyloliquefaciens*, be preserved in order for optimal promoter expression, as well as the knowledge of the importance of sequence length on promoter expression, one would not be motivated to extend the Shine-Delgarno sequence by the three nucleotides, TCC, of the present invention. Rather, one skilled in the art would be motivated to preserve such a sequence in order to preserve the original sequence of  $\alpha$ -amylase DNA promoters so as to ensure optimal promoter activity.

Applicants submit that the new and unexpected enhanced expression of the  $\alpha$ -amylase promoter derived from the microorganism *B. amyloliquefaciens* of the present invention is due directly to the novel nucleotide sequence, nucleotide length and position, i.e., 3' terminus, of the  $\alpha$ -amylase promoter, as attested to by the Declarant. Furthermore, Palva et al. and those skilled in the art neither teach nor suggest the specific modifications of nucleotide sequence, length and position of the  $\alpha$ -amylase promoter in the region of the critical Shine-Delgarno sequence of the  $\alpha$ -amylase promoter, which results in the above-described new and unexpected results of the present invention. Indeed, Palva et al. and common knowledge in the art actually teach away from the novel modifications of the  $\alpha$ -amylase promoter of the present invention, based on the long

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believed dogma that the conservation of the promoter structure in *B. subtilis* be preserved for optimal promoter expression.

For all the foregoing reasons, it is believed that amended claims 4 and 31 patentably define over the prior art. Withdrawal of the asserted rejections and allowance of all pending claims 4, 9-10, 15-16 and 31-35 is respectfully requested.

Applicants filed two Information Disclosure Statements dated January 14, 2002, and February 11, 2002. The Examiner returned an initialed copy of our February 11, 2002 Information Disclosure Statement, but we have not yet received an initialed copy of our January 14, 2002 Information Disclosure Statement. Applicants are providing herewith another copy of our Form PTO-1449 filed January 14, 2002, and ask that the Examiner initial and return the document for our records.

In addition, a Notice of Acceptance issued in this case on October 30, 2001, with an incorrect date of receipt. Applicants filed a Request for Corrected Notice of Acceptance on April 11, 2002, requesting that the date be corrected to read as -- 09/07/2001--. Applicants included a copy of our Express Mail receipt dated "SEP 7 2001" and a copy of our postcard date-stamped by the PTO Mail Room as having been received "07 SEP 2001". Please confirm that the date of receipt has been corrected in the records of the U.S. Patent and Trademark Office to indicate September 7, 2001.

Respectfully submitted,

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